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(51) International Patent Classification<sup>7</sup>: **C07K 19/00**,  
A61P 31/00 // C07K 14/245, 14/28, 14/235

Declarations under Rule 4.17:

— as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii)) for the following designations AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW, ARIPO patent (BW, GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG)

— as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii)) for the following designations AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW, ARIPO patent (BW, GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG)

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23 December 2004

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: MUTANT CHOLERA HOLOTOXIN AS AN ADJUVANT AND AN ANTIGEN CARRIER PROTEIN

(57) Abstract: The invention relates to a mutant cholera holotoxin having reduced toxicity, which functions both as an adjuvant and an antigen carrier. In a particular embodiment, the cholera holotoxin is genetically modified at least at amino acid residue 29 of the A subunit, wherein the genetic modification comprises an amino acid substitution of the wild-type glutamic acid at position 29, wherein the substitution is not an aspartic acid.

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# INTERNATIONAL SEARCH REPORT

International Application No  
PCT/US2004/007673

**A. CLASSIFICATION OF SUBJECT MATTER**  
IPC 7 C07K19/00 A61P31/00  
//C07K14/245, C07K14/28, C07K14/235

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)  
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS, PAJ, WPI Data, EMBASE

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category <sup>a</sup>	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	HAJISHENGALLIS G ET AL: "MUCOSAL IMMUNIZATION WITH A BACTERIAL PROTEIN ANTIGEN GENETICALLY COUPLED TO CHOLERA TOXIN A2/B SUBUNITS" JOURNAL OF IMMUNOLOGY, THE WILLIAMS AND WILKINS CO. BALTIMORE, US, vol. 154, no. 9, 1 May 1995 (1995-05-01), pages 4322-4332, XP000645280 ISSN: 0022-1767 abstract; figure 5	1-3, 6-15, 29-34, 52-54, 57, 59-66, 80,82-85
Y	----- -----	4,5, 16-28, 55,56, 58,67-79

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

<sup>a</sup> Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the International filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the International filing date but later than the priority date claimed

- "T" later document published after the International filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "&" document member of the same patent family

Date of the actual completion of the International search	Date of mailing of the International search report
18 October 2004	04.11.2004
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Authorized officer  Wagner, R

# INTERNATIONAL SEARCH REPORT

International Application No

PCT/US2004/007673

**C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT**

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 98/42375 A (CHIRON CORP) 1 October 1998 (1998-10-01) cited in the application  the whole document	4,5, 16-28, 36-43, 55,56, 58, 67-79, 87-94
Y	WO 97/02348 A (GIANNELLI VALENTINA ; PIZZA MARIAGRAZIA (IT); BIOCINE SPA (IT); RAPPNUO) 23 January 1997 (1997-01-23) cited in the application the whole document	4,5, 16-28, 55,56, 58,67-79
Y	WO 93/13202 A (SCLAVO BIOCINE SPA) 8 July 1993 (1993-07-08) cited in the application  the whole document	4,5, 16-28, 55,56, 58,67-79
Y	WO 00/18434 A (JOBLING MICHAEL G ; GREEN BRUCE A (US); PEEK JOEL A (US); US HEALTH (U) 6 April 2000 (2000-04-06)  the whole document	4,5, 16-28, 55,56, 58,67-79
X	MARTIN M ET AL: "Recombinant antigen-enterotoxin A2/B chimeric mucosal immunogens differentially enhance antibody responses and B7-dependent costimulation of CD4+ T cells" INFECTION AND IMMUNITY, AMERICAN SOCIETY FOR MICROBIOLOGY. WASHINGTON, US, vol. 69, no. 1, January 2001 (2001-01), pages 252-261, XP002231728 ISSN: 0019-9567 abstract	1,12,52, 63
X	JOBLING MICHAEL G ET AL: "Fusion proteins containing the A2 domain of cholera toxin assemble with B polypeptides of cholera toxin to form immunoreactive and functional holotoxin-like chimeras" INFECTION AND IMMUNITY, vol. 60, no. 11, 1992, pages 4915-4924, XP002292263 ISSN: 0019-9567 abstract	1,12,52, 63
A	US 6 395 964 B1 (ARNTZEN CHARLES J ET AL) 28 May 2002 (2002-05-28) column 17	1-34, 52-85
		-/-

# INTERNATIONAL SEARCH REPORT

International Application No

PCT/US2004/007673

**C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT**

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 93/07178 A (PASTEUR MERIEUX SERUMS VACC) 15 April 1993 (1993-04-15) page 10 - page 12; claim 10 -----	44, 95-101
X	WO 01/74383 A (UAB RESEARCH FOUNDATION) 11 October 2001 (2001-10-11) figure 1 -----	35,86
Y	GIANNELLI V ET AL: "Protease susceptibility and toxicity of heat-labile enterotoxins with a mutation in the active site or in the protease-sensitive loop." INFECTION AND IMMUNITY. JAN 1997, vol. 65, no. 1, January 1997 (1997-01), pages 331-334, XP002301115 ISSN: 0019-9567 page 331 -----	36-43, 87-94
X	SCHNEERSON R ET AL: "SYNTHESIS OF A CONJUGATE VACCINE COMPOSED OF PNEUMOCOCCUS TYPE 14 CAPSULAR POLYSACCHARIDE BOUND TO PERTUSSIS TOXIN" INFECTION AND IMMUNITY, AMERICAN SOCIETY FOR MICROBIOLOGY. WASHINGTON, US, vol. 60, no. 9, 1 September 1992 (1992-09-01), pages 3528-3532, XP000371779 ISSN: 0019-9567 abstract -----	44,95
X	WO 02/47727 A (WILSON ANDREW DOUGLAS ; ONG KONG WEE (GB); UNIV BRISTOL (GB); MORGAN A) 20 June 2002 (2002-06-20) claim 14 -----	35,86
X	CARBONETTI NICHOLAS H ET AL: "Stimulation of HIV gp120-specific cytolytic T lymphocyte responses in vitro and in vivo using a detoxified pertussis toxin vector" AIDS RESEARCH AND HUMAN RETROVIRUSES, vol. 17, no. 9, 10 June 2001 (2001-06-10), pages 819-827, XP002301116 ISSN: 0889-2229 page 820 -----	44-51, 95-101

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US2004/007673

### Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1.  Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
  
2.  Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
  
3.  Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

### Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1.  As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
  
2.  As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
  
3.  As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
  
4.  No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

#### Remark on Protest

The additional search fees were accompanied by the applicant's protest.

No protest accompanied the payment of additional search fees.

**FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210**

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-11, 12-34, 52-62, 63-85

Claims 1-11, 12-34, 52-62, 63-85 which are directed to an antigen covalently associated to a mutated cholera holotoxin (CT)  
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2. claims: 35-43, 86-94

Claims 35-43, 86-94, which are directed to an antigen covalently associated to an Escherichia Coli heat labile toxin (LT).  
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3. claims: 44-51, 95-101

Claims 44-51, 95-101, which are directed to an antigen covalently associated with a pertussis toxin (PT).  
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**INTERNATIONAL SEARCH REPORT**

Information on patent family members

International Application No

PCT/US2004/007673

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**INTERNATIONAL SEARCH REPORT**

Information on patent family members

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